Arch. Biol. Sci., Belgrade, 64 (1), 15-20, 2012 DOI:10.2298/ABS1201015N

INVESTIGATION OF ANTIBACTERIAL ACTIVITY OF CINNAMYL DERIVATIVES OF ARYLPIPERAZINE

IRENA NOVAKOVIĆ[1*], JELENA PENJIŠEVIĆ[1], V. ŠUKALOVIĆ[1], DEANA ANDRIĆ[2], G. ROGLIĆ[2] and SLAĐANA KOSTIĆ-RAJAČIĆ[1]

[1] *University of Belgrade, Center of Chemistry, ICTM,* 11000 Belgrade, Serbia
[2] *Faculty of Chemistry, University of Belgrade,* 11000 Belgrade, Serbia

Abstract - The derivatives of cinnamic acid and N-arylpiperazine show antibacterial activity. In this work the potential synergistic effect of cinnamyl derivatives of arylpiperazine in selected bacteria was investigated. The antibacterial activities of the derivatives were evaluated against Gram-positive bacteria: *Staphylococcus aureus*, *Streptosporangium longisporum*, *Sarcina lutea*, *Micrococcus flavus*, *Clostridium sporogenes* and *Bacillus subtilis* and Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Proteus vulgaris* by the disc diffusion method. The minimum inhibitory concentration (MIC) against the selected bacteria was determined for all compounds that showed activity in the disc diffusion method. The majority of the investigated compounds displayed *in vitro* antibacterial activity. The effect of the type and structure of the substituent on the aromatic ring on the antibacterial activity is discussed. It was found that two derivatives expressed activity toward *S. longisporum* and *P. aeruginosa* that was almost as strong as that of amikacin.

Key words: Arylpiperazine, antibacterial activity, minimum inhibitory concentration, *in vitro* studies

INTRODUCTION

Over the last 10-15 years, many pathogenic bacteria and parasites have become resistant to commercial chemotherapeutic agents. As a result, the investigation and development of potent and effective antibacterial agents represent one of the most important goals in pharmacy. However, at the moment only five major pharmaceutical companies, Astra Zeneca, GSK, Merck, Novartis and Pfizer, have active antibacterial drug discovery programs (Škedelj et al., 2011). The main problem with antibacterial drug discovery is the low rate of approval for clinical use that some of them experience, as well as the required time of 8-12 years it takes for an antibiotic to pass from discovery to the market (Tompson et al., 2004). Over the last 40 years only two new structural types of antibacterial drugs, daptomycin (lipopeptide in structure) and linezolid (oxazolinidone in structure) have been clinically used. It is well known that a number of heterocyclic compounds containing nitrogen and sulfur are associated with various types of biological activities. Piperazines and their analogs are a significant moiety that can be found in many marketed drugs, such as the Merck HIV protease inhibitor, Crixivan (Dorsey et al., 2000). Piperazinyl compounds are reported as being potent antibacterial agents against resistant strains (Kerns et al., 2002), antimalarial agents (Ryckebusch et al., 2003), dual calcium agonists (Kimura et al., 2002), and antipsychotic agents (Stahl and Grady, 2004). Also, cinnamic acid and its derivates play an important role in antibacterial activity (Narasimhan et al., 2004). We investigated the antibacterial activity of cynnamil-piperazine. All compounds reported in this paper were screened for their *in vitro* antibacte-

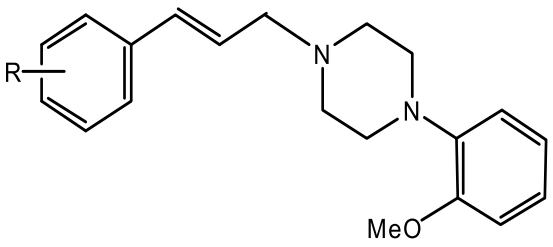

Table 1. Structural formulae of the derivatives.

Number	Name	R
1	(2*E*)-4-(2-methoxyphenyl)-1-(3-phenylprop-2-enyl)-piperazine	H
2	(2*E*)-4-(2-methoxyphenyl)-1-[3-(2-methoxyphenyl)prop-2-enyl]-piperazine	2-OCH_3
3	(2*E*)-4-(2-methoxyphenyl)-1-[3-(3-methoxyphenyl)prop-2-enyl]-piperazine	3-OCH_3
4	(2*E*)-4-(2-methoxyphenyl)-1-[3-(4-methoxyphenyl)prop-2-enyl]-piperazine	4-OCH_3
5	(2*E*)-4-(2-methoxyphenyl)-1-[3-(2-nitrophenyl)prop-2-enyl]-piperazine	2-NO_2
6	(2*E*)-4-(2-methoxyphenyl)-1-[3-(3-nitrophenyl)prop-2-enyl]-piperazine	3-NO_2
7	(2*E*)-4-(2-methoxyphenyl)-1-[3-(4-nitrophenyl)prop-2-enyl]-piperazine	4-NO_2
8	(2*E*)-1-[3-(2-chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)-piperazine	2-Cl
9	(2*E*)-1-[3-(3-chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)-piperazine	3-Cl
10	(2*E*)-1-[3-(4-chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)-piperazine	4-Cl

rial activity against Gram-positive and Gram-negative bacterial strains using the minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

The antibacterial activities of cinnamic acid, 1-(2-methoxyphenyl)-piperazine and cinnamyl derivatives of arylpiperazine (Table 1) were evaluated. All the derivatives were synthesized by the previously published method (Penjišević et al., 2007).

Antibacterial investigations

The antibacterial activities of the compounds were evaluated against six Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Streptosporangium longisporum* (ATCC 25212), *Sarcina lutea* (ATCC 9341), *Micrococcus flavus* (ATTC 10240), *Clostridium sporogenes* (ATCC 19404) and *Bacillus subtilis* (ATCC 6633) and against four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13076) and *Proteus vulgaris* (ATCC 13315).

Disc-diffusion method

A disc-diffusion method, according to the NCCLS, was employed for the determination of the antibacterial activity of the compounds (NCCLS 1997). Mueller Hinton agar was prepared according to the instructions of the manufacturer. All agar plates were prepared in 90 mm Petri dishes and 100 μL of a suspension of the tested microorganisms was spread on solid media plates. Sterile filter paper discs (6 mm in diameter) were impregnated with 50 μL of the sample solution in DMSO (giving 1000 μg per disc) and placed on inoculated plates. After standing at 4°C for 2 h, the plates were incubated at 37°C for 24 h. Standard discs of tetracycline (Institute of Immunology and Virology "Torlak": 30 μg of the active component, diameter 6 mm) were used as a positive control, while discs imbued with 50 μL of pure DMSO were used as a negative control. The diameters of the inhibition zones (including the disc) were measured in millimeters. Each test was performed in triplicate.

Table 2. *In vitro* antibacterial activity (mm) against Gram-negative bacteria at a concentration of 1000 μg/disc.

Compound	*E. coli*	*P. aeruginosa*	*S. enteritidis*	*P. vulgaris*
1	16	14	14	-
2	22	21	22	22
3	15	14	16	-
4	15	10	19	-
5	13	10	14	-
6	17	10	15	-
7	-	-	-	-
8	23	23	21	24
9	18	19	18	-
10	-	-	-	-
Cinnamic acid	17	17	14	16
1-(2-Methoxyphenyl)-piperazine	18	15	15	15
Tetracycline	26	25	25	26

- inactive
[a] concentration 30 μg/disc

Table 3. *In vitro* antibacterial activity (mm) against Gram-positive bacteria at a concentration of 1000 μg/disc.

Compound	*S. aureus*	*S. longisporum*	*S. lutea*	*M. flavus*	*C. sporogenes*	*B.subtilis*
1	15	14	13	15	-	-
2	23	24	20	24	20	22
3	16	14	16	14	-	-
4	18	16	16	16	-	-
5	14	13	15	20	-	-
6	15	17	16	17	-	-
7	-	-	-	-	-	-
8	25	24	25	24	21	21
9	24	18	17	18	-	-
10	-	-	-	-	-	-
Cinnamic acid	14	16	19	21	14	14
1-(2-Methoxyphenyl)-piperazine	16	15	15	15	16	15
Tetracycline[a]	24	24	23	27	24	25

- inactive
[a] concentration 30 μg/disc

Determination of the minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was performed by the broth microdilution method in 96-well microtiter plates according to the NCCLS (NCCLS 2000). Sterile 96-well polystyrene microtiter plates with well capacities of 300 μl were used and 100 μl of fresh Mueler-Hinton broth was added to each well of the plate. One hundred μL of the stock solution of the tested compounds in DMSO (concentration about 2 mg/100 μL) was added to the wells of the first column. Then 100 μl of the solution were removed from the first column and mixed thoroughly with the broth in the corresponding wells of the second column six times. Subsequently, a 100 μL aliquot was removed from each well in this column and mixed with the broth in the corresponding well of the next column. This doubling dilution was performed in rows across the plate. The same procedure was repeated with stock solutions of each of the tested compounds. In the last

Table 4. The MIC value (mg/mL) of the active compounds against Gram-negative bacteria.

Compound	*E.coli*	*P. aeruginosa*	*S. enteritidis*	*P. vulgaris*
1	1.4	2.8	2.8	-
2	0.05	0.05	0.05	0.05
3	2.8	2.8	1.4	-
4	1.4	5.6	0.7	-
5	2.8	5.6	1.4	-
6	2.8	5.6	2.8	-
8	0.05	0.05	0.1	0.05
9	0.2	0.2	0.2	-
Cinnamic acid	0.36	0.36	2.88	1.44
1-(2-Methoxyphenyl)-piperazine	0.2	1.6	1.6	1.6
Amikacin	0.004	0.044	0.009	0.008

- inactive

Table 5. The MIC value (mg/mL) of the active compounds against Gram-positive bacteria.

Compound	*S. aureus*	*S. longisporum*	*S. lutea*	*M. flavus*	*C. sporogenes*	*B. subtilis*
1	1.4	2.8	2.8	1.4	-	-
2	0.05	0.05	0.1	0.05	0.1	0.05
3	1.4	2.8	1.4	2.8	-	-
4	0.7	1.4	1.4	1.4	-	-
5	2.8	2.8	1.4	0.35	-	-
6	2.8	1.4	2.8	1.4	-	-
8	0.05	0.05	0.05	0.05	0.1	0.1
9	0.05	0.2	0.4	0.2	-	-
Cinnamic acid	2.88	1.44	0.72	0.09	2.88	2.88
1-(2-Methoxyphenyl)-piperazine	1.6	1.6	1.6	1.6	1.6	1.6
Amikacin	0.015	0.058	0.003	0.004	0.017	0.031

- inactive

row, double dilution was performed with pure DMSO solution and this row was used as control. Ten μL of the bacteria cultures was inoculated into each well of rows of the plate. The microtiter plate was incubated at 37°C for 24 h, after which the bacterial growth was measured. The MIC was determined as the lowest concentration that resulted in inhibition of bacterial growth. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

The antibacterial activities of cinnamic acid, 1-(2-methoxyphenyl)-piperazine and cinnamyl derivatives of arylpiperazine were first tested by the agar disc diffusion method against Gram-positive and Gram-negative bacteria. The results of these studies, represented as zones of inhibition, are summarized in Tables 2 and 3.

As can be seen from the data, both cinnamic acid and 1-(2-methoxyphenyl)-piperazine expressed activity against all investigated bacteria cells. We assumed that cinnamyl derivatives of aryl-piperazine would exhibit antibacterial activity. It was found that most of the synthesized derivatives displayed *in vitro* antibacterial activity against selected bacteria. Some derivatives showed a better activity than the starting compounds. These results indicated a potential synergistic antibacterial effect of synthesized derivatives. Compared with compound **1**, introduction of

new groups in the aromatic moiety of the cinnamic acid in some cases provided derivatives with a better antibacterial activity.

The main physiological differences between Gram-positive and Gram-negative bacteria are the structure of their cell walls. The outer layer of the outer membrane of Gram-negative bacteria is composed primarily of lipopolysaccharide molecules, while a thin peptidoglycan layer is situated below the lipopolysaccharide layer. In this way, Gram-negative bacteria are protected against the effect of drugs.

Gram-positive cell walls typically lack the outer membrane found in Gram-negative bacteria. In Gram-positive bacteria, the plasma membrane is attached to the thick peptidoglycan layer. Therefore, these bacteria are sensitive to the effects of lysozyme and hydrophilic drugs.

Since the outer layer of Gram-negative bacteria is negatively charged, it was expected, that most of the cinnamyl derivatives of arylpiperazine with delocalized positive charge at the cinnamic moiety, would show a better activity toward Gram-negative bacteria, which was indeed observed, except in the case of *P. vulgaris*. The best results were obtained by introducing substituents with positive resonance effect in position 2 of aromatic moiety, e.g. **2** and **8**. The derivative **4** possessed a moderate activity while derivatives **7** and **10** did not show any antibacterial activity. It seems that introduction of a substituent at position 4 afforded derivatives with weak or no antibacterial activity. Derivative **9** showed significant activity toward most of the selected bacterial strains. The derivatives with a substituent with a negative resonance effect on aromatic moiety, **5** and **6** expressed a weak activity against Gram-negative bacteria.

Against the selected Gram-positive bacteria, the derivatives **2** and **8** were highly active. As with the Gram-negative bacteria, derivative **9** expressed good activity. Derivatives **7** and **10** did not show any antibacterial activity. Other derivatives displayed a moderate activity against all selected bacteria cells, except *C. sporogenes* and *B. subtilis*. It was observed that, as in the cases of the Gram-negative bacteria, the most active were those derivatives that have a substituent with a positive resonance effect on the aromatic ring.

In the second phase, the determination of the MIC was performed by the broth microdilution method. This determination was performed with all derivatives that showed antibacterial activity that was observed in the disc diffusion method. The results are presented in Tables 4 and 5.

As can be seen from the data, the MIC values are in accordance with the results obtained by the disc diffusion method. Compound 1-(2-methoxyphenyl)-piperazine showed a weak activity against all selected bacteria (1.6 mg/mL) apart from *E. coli* (0.2 mg/mL), while cinnamic acid in most cases showed a moderate activity and the best activities were towards *M. flavus*, *E. coli*, *P. aeruginosa* and *S. lutea* (0.09-0.72 mg/mL). Compared with these compounds, some of the synthesized cinnamyl derivatives of arylpiperazine gave a better activity against the bacteria strains. The most active derivatives were **2** and **8**. Against bacteria *S. longisporum* and *P. aeruginosa* these derivatives showed an *in vitro* activity almost as strong as amikacin (0.05 mg/mL). Derivative **9** expressed a good antibacterial activity. The range of MIC values for this derivative was 0.05-0.2 mg/mL. Toward both Gram-positive and Gram-negative bacteria, the derivatives **3**, **4**, **5** and **6** showed weak activity, while against *C. sporogenes, B. subtilis* and *P. vulgaris* these derivatives had no activity.

CONCLUSIONS

In this work the antibacterial activities of cinnamic acid, 1-(2-methoxyphenyl)-piperazine and cinnamyl derivatives of arylpiperazine were investigated. Most of the synthesized compounds displayed *in vitro* antibacterial activity against most Gram-positive and Gram-negative bacteria. The best results were observed for the derivatives (2*E*)-4-(2-methoxyphenyl)-1-[3-(2-methoxyphenyl)prop-2-enyl]-piperazine (**2**) and (2*E*)-1-[3-(2-chlorophenyl)prop-2-enyl]-4-(2-methoxyphenyl)-piperazine (**8**). These derivatives

showed the highest activity toward all investigated bacteria cells, while against *S. longisporum* and *P. aeruginosa* they had an *in vitro* activity which was almost as strong as amikacin (0.05 mg/mL).

By comparing the antibacterial activity of all the synthesized derivatives, it was found that both the type of the substituent and its position on the aromatic ring had a significant impact on activity in bacterial strains. It was observed that position 2 on the aromatic ring gave derivatives with the highest activity, while the substituent in position 4 gave derivatives with weak or no activity. The best results were obtained when in position 2 on the aromatic ring there were substituents with a positive resonance effect. The electron acceptor group, in this case the nitro group, decreased the general antibacterial activity regardless of whether the group was in position 2, 3 or 4 on the aromatic ring. It may be concluded that the antibacterial activity of the synthesized compounds is related to the structure of the cell walls of the bacteria.

Acknowledgements- These results are part of Project 172032, supported by the Ministry of Education and Science of the Republic of Serbia.

REFERENCES

Dorsey, B. D., McDonough, C., McDaniel, S. L., Levin, R. B., Newton, C. L., Hoffman, J. M., Darke, P. L., Zugay-Murphy, J. A., Emini, E. A., Schleif, W. A., Olsen, D. B., Stahlhut, M. W., Rutkowski, C. A., Kuo, L. C., Lin, J. H., Chen, I-W., Michelson, S. R., Holloway, M. K., Huff, J. R., and *J. P. Vacca* (2000). Identification of MK-944a: A second clinical candidate from the hydroxylaminepentanamide isostere series of HIV protease inhibitors. *J. Med. Chem*. **43**, 3386-3399.

Kerns, R. J., Rybak, M. J., Kaatz, G. W., Vaka, F., Cha, R., Grucz, R. G., and *V. U. Diwadkar* (2002). Synthesis of some novel piperazine salts and their antimicrobial property against *Escherichia coli* and *Bacillus subtilisi. Bioorg. Med. Chem. Lett*. **12,** 3111-3115.

Kimura, M., Masuda, T., Yamada, K., Kobuta, N., Kawakatsu, N., Mitani, M., Kishii, K., Inazu, M., Kiuchi, Y., Oguchi, K., and T. Namiki (2002). Novel diphenylalkyl piperazine derivatives with dual calcium antagonistic and antioxidative activities. *Bioorg. Med. Chem. Lett.* **12**, 1947-1950.

Narasimhan, B., Belsare, D., Pharande, D., Mourya, V., and *A. Dhake* (2004). Esters, amides and substituted derivatives of cinnamic acid: synthesis, antimicrobial activity and QSAR investigations. *Eur. J. Med. Chem.* **39**, 827-834.

NCCLS (National Committee for Clinical Laboratory Standards) (1997). Performance standards for antibacterial disk susceptibility test, 6th edn. Approved Standard M2-A6, Wayne, PA

NCCLS (National Committee for Clinical Laboratory Standards) (2000). Approval standard document M7-A5, Vilanova, Pa, USA

Penjišević, J., Šukalović, V., Andrić, D., Kostić-Rajačić, S., Šoškić, V., and *G. Roglić* (2007). 1-Cinnamyl-4-(2-methoxyphenyl)piperazines: Synthesis, Binding Properties, and Docking to Dopamine (D_2) and Serotonin ($5\text{-}HT_{1A}$) Receptors. *Arch. Pharm. Chem. Life Sci*. **340**, 456-465.

Ryckebusch, A., Poulain, R., Maes, L., Debreu-Fontaine, M. A., Mouray, E., Grellier, P., and *C. Sergheraert* (2003). Synthesis and in vitro and in vivo antimalarial activity of N^1-(7-Chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine derivatives. *J. Med. Chem.* **46**, 542-557.

Stahl, S. M., and *M. M Grady* (2004). A critical review of atypical antipsychotic utilization: Comparing monotherapy with polypharmacy and augmentation. *Curr. Med. Chem.* **11,** 313-327.

Thompson, C. J., Power, E., Ruebsamen-Waigmann, H., and *H. Labischinski* (2004). Antibacterial research and development in the 21(st) Century - an industry perspective of the challenges. *Curr. Opinion Microbiol.* **7**, 445-450.

Škedelj, V., Tomašić, T., Peterlin Mašič, L., and *A. Zega* (2011). ATP-binding site of bacterial enzymes as a target for antibacterial drug design. *J. Med. Chem.* **54**, 915-929.